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Evaluating Rice Straw as a Substitute for Barley Straw in Inhibiting Algal Growth in Farm Ponds

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Abstract

Algal blooms disrupt aquatic ecosystems and are more common in lakes, ponds, and rivers during the summer months due to nutrient pollution. Livestock production can contribute increased quantities of nutrients to water bodies from runoff of manure. Commonly used mechanical and chemical control methods may have limited success because algae are small and propagate quickly. Barley (*Hordeum vulgare*) straw has been shown to inhibit the growth of algae as the straw decomposes aerobically in ponds. Therefore, barley represents a natural option for algal biomass control. However, the small amount of barley production in Arkansas limits the availability of barley straw as a solution to control algal blooms locally. Other cereal grain straws may produce similar inhibitory effects during decomposition. Rice (*Oryza sativa*) is produced in large quantities in Arkansas, making rice straw a locally sourced straw product. The objective of this research was to determine the efficacy of using rice compared to barley straw to inhibit algal growth in freshwater ponds. Data were collected from nine farm ponds, three treated with rice straw, three treated with barley straw, and three without amendment to serve as the experimental control. Dissolved oxygen, pH, nitrate-nitrogen (NO_3^--N), dissolved phosphorus (P), temperature, and turbidity were measured for 14 weeks from June 12 to September 17, 2018. Algal biomass was measured as chlorophyll-a concentration to evaluate treatment effectiveness over time. Dissolved oxygen was significantly influenced by treatment and time. The NO_3^--N concentration in ponds treated with rice straw was significantly greater than the control and barley treatment. Chlorophyll-a concentrations were variable, and there were no consistent trends through time within a treatment. More research under controlled conditions to understand impacts of abiotic conditions, microbial and algal community compositions, and mode of action of algal inhibition is required before cereal straw can be a reliable, locally sourced method of algal control in farm ponds.

Introduction and Literature Review

Algae are present in almost every aquatic ecosystem, play a key ecological role through photosynthesis, and serve as a food source for higher trophic levels. Phytoplankton are free-floating algae that live in the epilimnion that can grow to large numbers forming algal blooms. These algal blooms, or elevated densities of algal populations, compromise ecosystem health. Increased nutrient concentrations from human activities, such as fertilizer use and livestock production, contribute to more frequent algal blooms (Islami and Feliziadeh, 2011). The increase in algal abundances can turn the water color, commonly green in freshwater, and can cause a foul odor. Additionally, dissolved oxygen becomes limited as the algae die and decompose (Kannan and Lenca, 2012). Blue-green algae, while grouped with algae, are photosynthetic bacteria called cyanobacteria (Kannan and Lenca, 2012). Blue-green algae can turn the water green, produce a foul odor, and release cyanotoxins that may be harmful to humans and animals. Attempts to control algae are rarely successful because algae are small and reproduce quickly. Mechanical removal is inefficient and must be repeated periodically, while treatment with chemical algicides can harm non-target organisms (Swistock, 2017).

Barley (*Hordeum vulgare*) straw can be used as an alternative method of algal control. As barley straw decomposes aerobically, barley straw releases a chemical, or combination of chemicals, that inhibits the growth of green algae and cyanobacteria without harming other aquatic life (Islami and Feliziadeh, 2011). While the precise inhibitory chemical is not known, it has been hypothesized that weak peroxides and oxidized polyphenols are responsible for algal growth inhibition (Islami and Feliziadeh, 2011). Straw must be placed in ponds 2 to 8 weeks before the algal growing season, depending on water temperature, to give the straw time to begin decomposing (Lembi, 2002). Maximum toxicity to blue-green algae occurs after one month of decomposition and declines over the following months until decomposition is complete (Rice et al., 1980). Decomposition of the barley straw may decrease dissolved oxygen, but the lack of competition for light from algae allows more photosynthesis from higher-order plants (Newman, 2004).

While barley straw has potential to be an environmentally "clean" form of algal control, there are some concerns regarding the adoption of barley straw for algal control. Barley straw acts as an algistat, rather than an algicide, such that barley straw does not kill existing algal cells but prevents the growth of more algae. Because the Environmental Protection Agency has not certified barley as an algistat, barley straw can be marketed legally only as a home remedy for preventing algal growth (Lembi, 2002). A logistical challenge to using barley straw in Arkansas is that barley is not a commonly cultivated crop (United States Department of Agriculture, 2018). Barley production in the United States is concentrated in the northern midwestern and northwestern states, such as North Dakota, Montana, and Washington, rendering barley straw unavailable to much of the country (Guercio, 2018).

Arkansas is the largest rice (*Oryza sativa*) producer in the country based on planted area (United States Department of Agriculture, 2018). Though studies using cereal straw to prevent algal growth have concentrated on barley straw, other cereal grain straws may be effective substitutes for barley straw because similar chemicals are produced during decomposition (Newman, 2004; Park et al., 2006). The large quantities of rice straw in Arkansas make rice straw favorable when attempting to minimize cost of algal control. Furthermore, cyanobacterial populations in rice paddies were less dense in the second year of cultivation than in the first year when residues from the first year were left in the paddies, lending support to the hypothesis that rice straw is effective at inhibiting algal growth (Rice et al., 1980).

Research Question

1. Is barley straw effective at inhibiting algal growth in farm ponds in Northwest Arkansas?
2. Is rice straw an adequate substitute for barley straw in algal bloom inhibition?

Objective

The objective of this research project was to determine if rice straw is as effective as barley straw at preventing the growth of algae when allowed to decompose aerobically in ponds during the algal growing season.

Prediction

Algal growth, measured as chlorophyll-a, is expected to be reduced in ponds with rice and barley straw when compared to an untreated control. Rice straw is expected to be as effective as barley straw at preventing the growth of algae, measured as chlorophyll-a.

Materials and Methods

Experimental Setup

After presenting the project background, research questions, and anticipated experimental approach to the Ozark Cattlemen's Association and faculty, five volunteers agreed to participate in the project by granting access to their ponds. Nine farm ponds in Washington County were selected for this experiment (Figure 1). For two properties, three ponds were located on one property and each treatment was randomly assigned to a pond on the property. For the last three remaining ponds located on different properties, each treatment was assigned randomly to a pond (Figure 1). Ponds treated with barley straw were labeled pond B1, B2, and B3. Ponds treated with rice straw were labeled pond R1, R2, and R3, and the three ponds left untreated as an experimental control were labeled pond C1, C2, and C3. Surface area was calculated for all ponds by measuring the length and width with a tape measure of each pond. Ponds with straw were treated at a rate of 25 g/m^2 (Abou El Ella et al., 2007).

The appropriate masses of barley and rice straw were portioned for the respective ponds, cut into pieces approximately 15 cm in length, and placed into plastic 0.5-cm mesh bags. Bags were packed loosely, so water could easily flow through the bag and contact the decomposing straw. When filled with straw, bag volume approximated 90 cm by 55 cm by 40 cm. Pool noodles were tied to the bags with twine to keep the bags afloat in the ponds and promote conditions for aerobic decomposition. Bags were placed on their sides, so that the bottom of the bag was at a depth of approximately 20 cm. Each straw bag was anchored to the pond floor using bricks tied to the ends of string stabilizing placement and evenly spacing bags within ponds. Due to varying pond size, number of straw bags per pond ranged from one bag in pond R1to eight bags in pond B2. Brick anchors were attached by a length of string equal to the pond depth at the location of each straw bag. Barley and rice straw bags were placed in ponds on June 12, 2018.

Sampling

Beginning on June 12, 2018, when the rice and barley straw were placed in each respective pond, water samples were collected weekly from each of the nine ponds for 14 weeks. Ponds were sampled in the order: R1, B1, C1, R2, B2, R3, B3, C3, C2. Composite samples consisted of five individual 125-mL samples collected at a depth of 15 cm (625-mL total sample) at regular intervals across a transect dissecting each pond. Individual sample locations corresponded to the following: 1) close to the pond bank, 2) a quarter of the distance across the pond, 3) the center of the pond, 4) three quarters of the distance across the pond, and 5) at the opposite bank. Samples were collected traversing each pond in an aquatic sampling vessel to prevent water and sediment disturbance. Samples were immediately covered with aluminum foil to prevent further photosynthesis and photodegradation. The final date of sampling was September 17, 2018.

Chlorophyll-a

To measure chlorophyll-a concentrations, 50 mL from each composite pond sample were filtered in the field using a hand pump and GF/F filter (Whatman, 0.7-µm pore size). Filtrate was saved for further filtration for NO_3^--N and phosphorus analysis. After returning to the laboratory, each filter was soaked in 7 mL of 90% acetone for 24 hours and stored in a freezer. The extract was analyzed using a Trilogy Laboratory Fluorometer (Turner Designs, San Jose, CA). The "Chl-a" module of the fluorometer was calibrated using a stored calibration curve. After samples had been equilibrated to room temperature, extract from each sample (3 mL) was pipetted into a culture tube. Each tube was placed into the fluorometer one at a time. The sample was measured before acidification. After the measurement was complete, 0.1 mL of 0.1 N hydrochloric acid was pipetted into the tube. Following a 90-second reaction period, the sample was measured after acidification. The acidification step converts chlorophyll-a to pheophytin, a degradation product of chlorophyll-a, for conversion to a pheophytin-corrected chlorophyll-a concentration measured by the fluorometer. Resulting chlorophyll-a (µg/L) concentrations were recorded.

On week 14 (September 17, 2018), nine 50-mL water samples were collected at a depth of 15 cm in pond B2 to evaluate spatial distribution of chlorophyll-a concentrations. Water samples were collected at distances of 0, 3, and 6 m from three separate straw bags for a total of nine samples. Each sample was filtered in the field, prepared, and measured for chlorophyll-a concentration as described previously.

Nitrate-Nitrogen (NO_3^--N) and Dissolved Phosphorus

Using a 0.45-µm pore size nylon syringe filter (Cole Parmer, Vernon Hills, IL), 10 mL of filtrate from the chlorophyll-a procedure were filtered before leaving the field. Two drops of 5M hydrochloric acid (HCl) were added to each sample after filtration to preserve the sample. Nitrate-N concentrations were measured using cadmium reduction and the modified Griess reaction on a Sans-plus segmented-flow autoanalyzer (Skalar Inc, Buford, GA) (Baker et al., 2018). The calibration curve was prepared from 0, 1, 2, 5, 8, and 10 mg/L standards. The filtered and acidified water samples that did not produce an instrument response were recorded as a concentration of 0 mg NO_3^--N/L.

Filtered and acidified water samples were sent to the Agriculture Diagnostic Laboratory at the University of Arkansas to be analyzed for dissolved phosphorus on a Spectro Arcos inductively coupled plasma-optical emission spectrometer (ICP-OES) (SPECTRO Analytical Instruments, Kleve, Germany).

Water Quality Parameters

Dissolved oxygen was measured in-situ at each of the five locations along the sampling transect, at a depth of 15 cm, using a Lab Quest 2 (Vernier Software & Technology, Beaverton, OR) and Vernier Dissolved Oxygen Probe, and values were averaged for each pond. The pH, temperature, and turbidity were measured on the composite samples in the field using a pH Sensor, Stainless Steel Temperature Probe, and Turbidity Sensor (Vernier Software & Technology, Beaverton, OR).

Decomposition

To measure the decomposition of straw in each pond, the initial dry weight of straw was measured before adding to each pond. After the 14 weeks, the straw was removed from the ponds, dried in drying ovens at 55°C for three weeks, and weighed again. Percent decomposition was calculated by subtracting the final weight from the initial weight, dividing the difference by the initial weight and multiplying by 100.

Precipitation

Precipitation data were obtained from the Town Branch at Armstrong St. weather station in Fayetteville, AR on the United States Geological Survey website (United States Geological Survey, 2018).

Data Analysis

Chlorophyll-a concentrations were converted to relative percent difference from week 1 concentrations for each pond according to Equation 1.

$$\frac{x-x_0}{(\frac{x+x_0}{2})} * 100 \qquad (1)$$

where x_o was the chl-a value in week 1 and x was the chl-a value of the current week.

Averages, standard deviations, and standard error of the mean were calculated each week for the average relative percent difference in chlorophyll-a from week 1, NO_3^--N, phosphorus, dissolved oxygen, pH, temperature, and turbidity.

- $SE = \frac{\alpha}{\sqrt{n}}$

Data organization, graph creation, and data analysis were conducted in Excel 2016 (Microsoft Corp., Redmond, WA). Repeated measures analysis of variance (ANOVA) tests were performed on each dependent variable to determine statistical significance at $\alpha = 0.05$. Bonferroni post-hoc analysis was conducted on variables with significant *p*-values. Statistical analyses were used to determine if dependent variables differed across treatments over time. A t-test was used to determine statistical significance of straw decomposition between rice and barley straw ($\alpha = 0.05$). A single factor ANOVA was used to determine statistical significance among chlorophyll-a concentrations sampled at increasing distances from the straw bag ($\alpha = 0.05$). Linear regression was used to determine if dissolved oxygen and temperature changed through time at a 95% confidence level.

Results and Discussion

During the 14 weeks of this study, 28.5 ± 19.3% (average ± standard deviation) of the barley straw placed in ponds decomposed, while 43.7 ± 13.4% of the rice straw decomposed. Decomposition was not significantly different ($P = 0.2615$). Barley straw decomposed to the same extent as rice straw (~40%) in two of the ponds; however, pond B3 resulted in only a 6.7% decrease in barley straw. The dissolved oxygen was consistently low, usually between 3 and 4 mg/L in pond B3. The range for dissolved oxygen concentration in ponds with rice straw and the control was 4.1 mg/L to 6.5 mg/L and 3.9 mg/L to 6.7 mg/L, respectively (Figure 2). Pond B3 also had a layer of accumulated leaf litter on the bottom of the pond; therefore, aerobic microbial activity in B3, and thus aerobic decomposition of barley straw and production of any allelopathic compounds, may have been more constrained by abiotic conditions in the pond compared to other ponds.

Dissolved oxygen concentration differed significantly among the rice straw and barley straw treatments and the control ($P = 2.9\text{x}10^{-5}$). Time had a significant effect on dissolved oxygen ($P = 0.019$). Dissolved oxygen concentration in the barley straw treatment (4.26 ± 0.65 mg/L average ± standard deviation) was significantly different than dissolved oxygen in both the rice straw treatment (5.44 ± 0.73 mg/L average ± standard deviation) and the control (5.47 ± 0.76 mg/L average ± standard deviation). Dissolved oxygen concentration in week 1 (June 12, 2018) differed from weeks 4, 5, 7, and 8. Dissolved oxygen concentration in week 2 (June 18, 2018) differed from weeks 4, 5, and 8, and week 4 differed from weeks 11, 12, and 13.

Significantly lower dissolved oxygen concentration in the barley straw treatment could decrease the decomposition rate of barley straw in ponds. Although the average dissolved oxygen concentrations varied during the study, the average concentrations remained above 3.6 mg/L, which is sufficient for aerobic decomposition to occur (Cech, 2010). The differences in dissolved oxygen over time could be caused by the changes in water temperatures as the summer progressed. There could have been temporal or spatial locations in at least some of the ponds in which low oxygen concentrations were limiting to the efficacy of straw decomposition to control algal growth.

Initial chlorophyll-a concentrations ranged from 18.8 µg/L in pond B1 to 457 µg/L in pond R2 (Figure 3). Within the rice treatment, the initial range of concentrations was 436 µg/L. Ponds treated with barley had an initial range of 255 µg/L, and control ponds had an initial concentration range of 108 µg/L across the three ponds. These concentrations were much greater than those measured in the preliminary study (Appendix 1). Relative percent differences in chlorophyll-a concentrations from week 1 fluctuated through time in all treatments (Figure 4).

In week 14, the final sample date, both treatments and the control had negative relative percent differences from the week 1 concentration, meaning that there was less algal biomass in week 14 than week 1 in both treatments and the control. In the rice treatment, relative percent difference ranged from -90.1 to 69.8%. In the barley straw treatment, relative percent differences ranged from -119 to 23.7%. In the control group, relative percent differences raged from -127 to 80.2%. The relative percent difference in chlorophyll-a from week 1 of sample collection did not differ statistically between the straw treatments or between the treatments and the control (P = 0.845, Table 2). During the 14-week study, there were seven weeks when relative percent differences in all treatments were negative, meaning average chl-a concentrations were less than week 1, two weeks when the rice straw group was positive, meaning average chl-a showed growth compared to week 1, three weeks when the barley straw group was positive, and four weeks when the control group was positive. Thus, there was no indication of consistent control of algal biomass in either straw treatment, nor was there any consistent trend with algal biomass growth throughout the 14-week experiment (P = 0.694, Table 2).

The variability in chlorophyll-a among ponds within the same treatment could have been caused by environmental factors, such as pond sediment composition, the type and proximity of livestock to the ponds (Table 1), or the flow rate of water within the ponds, factors that were not quantified in this study. For example, pond R2 was spring-fed and feeds into an ephemeral stream. Relative percent difference in chlorophyll-a concentration was negative in all weeks after week 2 (Appendix 2), indicating algal inhibition throughout the study in pond R2 containing rice straw despite ducks, geese, and cattle having direct access to the pond (Table 1). The movement of water flowing across the pond may have circulated inhibitory chemicals from the decomposing straw throughout the pond. Abou El Ella et al. (2007) controlled algal growth with cereal straw in the Suez Canal, where wave and wind action caused consistent mixing of the water. However, other studies have shown that cereal straw is effective in lentic pond systems as well (Islami and Feliziadeh, 2011). Therefore, the efficacy of cereal straw to inhibit algal growth was not expected to be dependent on circulation of water; however, diffusion of inhibitory compounds within farm ponds may be a consideration that requires further investigation.

There was no statistical difference in chlorophyll-a concentrations with distance from the straw bags as measured at 0, 3, and 6 m in pond B2 on week 14 (September 17, 2018) (P = 0.495). Average chlorophyll-a concentrations were 41.98 ± 10.76, 41.86 ± 15.63, 52.78 ± 9.30 μg/L (average ± standard deviation) at 0, 3, and 6 m distance from straw bags, respectively. Lack of significance among chlorophyll-a concentrations at different distances from straw bags indicates that diffusion of chemicals dissipating away from the decomposing straw source was not the limiting factor to efficacy of straw as an algal growth inhibitor in the pond environment.

Water temperature was measured as a factor that could influence decomposition, the algal community, and dissolved oxygen concentrations. Both the treatment (P = 0.0093, Table 2) and time ($P = 2.85 \times 10^{-10}$) significantly affected water temperature (Figure 5). There was no difference among temperatures in the rice and barley treatments, however both the rice and barley treatments differed significantly from the control. Water temperature in week 2 (June 18, 2018) differed significantly from water temperature in week 13. Water temperature in week 3 (July 7, 2018) differed significantly from weeks 7, 10, and 13, week 4 (July 16, 2018) differed from weeks 7 and 13, and week 8 (August 6, 2018) differed from water temperature in week 13, respectively. Water temperature in week 11 (August 27, 2018) differed from weeks 1, 5, 6, 7, 9, 10, and 13, week 12 (September 5, 2018) differed from weeks 7, 9, 10, and 13, week 14 (September 17, 2018) differed from water temperature in weeks 7, respectively. The greater temperature in the control ponds could have been due to surrounding land management or an artifact of the sampling procedure. Control ponds lacked tree cover on the banks. More direct sunlight would increase water temperature. The last two ponds sampled each day were both in the control group, so the ponds had more time to warm throughout the day. Randomizing the order in which the ponds were sampled would have controlled for the effect time of day on water temperatures; however, sampling order was chosen using the most efficient route between ponds to assure all samples could be collected on the same day.

Straw treatment significantly affected NO_3^--N (P = 0.0003) and dissolved phosphorus (P = 0.024) concentrations, but concentrations did not differ significantly across sampling times (P = 0.976 for NO_3^--N and P = 0.274 dissolved P measurements across time, respectively, Table 2). Ponds containing rice straw had greater concentrations of NO_3^--N than ponds containing barley straw or the control (Figure 6). Average NO_3^--N concentrations in ponds containing barley straw and the control were 0.013 and .009 mg/L respectively. Average NO_3^--N concentrations in rice straw-treated ponds were 0.599 mg/L. In the rice straw treatment, average dissolved phosphorus concentration was 0.097 mg/L and did not differ significantly from the control which averaged 0.031 mg/L (Figure 7). In the barley straw treatment, average phosphorus concentration was 0.123 mg/L, which was significantly greater than the control.

Differences in NO_3^--N concentrations among treatments could have been due to the type and proximity of livestock to ponds. Cattle had access to all ponds (Table 1). Pond C2 had chickens roaming near the pond; although, the chicken house was downslope from the pond. Horses and donkeys were in fields adjacent to ponds R1, C1, and B1, but were never observed in the water on sampling dates. Pond R2 had domestic ducks and geese that nested on the bank of the pond, and pond R3 occasionally had wild ducks feeding in the pond. The waterfowl in ponds R2 and R3 might explain the greater concentration of NO_3^--N in the rice straw treatment. Low NO_3^--N levels in the control and barley straw ponds could indicate that the conditions necessary for algal growth were not present. The ideal nitrate-to-phosphate ratio by mass for algal growth is approximately 10:1, and concentrations of individual nutrient requirements vary among algal species (Downing and McCauley, 1992). During no week in either treatment or the control was NO_3^--N concentration great enough to achieve the ideal 10:1 nitrate-to-phosphate ratio for algal growth. Nutrient availability may have contributed to the lack of statistical differences in chlorophyll-a concentrations.

While not a statistically significant increase in NO_3^--N, observationally, NO_3^--N appeared to increase in the rice straw treatment in week 10 (August 21, 2018). Both the rice straw and barley straw treatment groups also appeared to show increases in average phosphorus concentration from week 9 (August 13, 2018) to week 10 (August 21, 2018), but this change in concentration was not significant. Phosphorus concentration in the control remained constant at 0.007 mg/L. This apparent increase in nutrient concentrations coincided with heavy rain during the preceding week prior to sample collection (Figure 7). The Washington County area received 7.77 cm of rain during week 10 of the experiment, which was the most of any week during the sample period (United States Geological Survey, 2018). The heavy rain could have resulted in more runoff and washed additional nutrients into the ponds (Daniel et al., 1994). In the week 11, following the spike in NO_3^--N, average relative percent difference in chlorophyll-a concentration in the rice straw group increased from -90.1% of the week 1 value to -16.8% (Figure 4). The increase in chlorophyll-a concentration, which had been declining slowly, may have resulted from increased uptake of available nutrients carried into the ponds with runoff. Algal uptake of nutrients could explain a lack of significant increase in nitrate-N and dissolved P concentrations in week 10.

There was no statistical difference in water pH among treatments and the control (P = 0.090, Table 2) and no significant effect of time (P = 0.202, Table 2). The average pH value was slightly acidic at 6.26 ± 0.17 (average ± standard deviation) (Appendix 3). As algal populations photosynthesize, carbonic acid, the dissolved form of carbon dioxide, is removed from the water, raising the pH (National Oceanic and Atmospheric Administration, 2017). The slightly acidic nature of the average pH levels suggests that there was no increase in algal populations impacting the pH in any of the treatment groups.

There was no statistical difference in turbidity among treatments and the control (P = 0.08, Table 2). Average turbidity was 52.28 ± 11.43 (average ± standard deviation) Nephelometric Turbidity Units (NTU) within both straw treatments and 45 and 75 NTU within the control group (Appendix 3). While not significant, average turbidity in all treatments appeared to be elevated during weeks with large precipitation amounts but not by more than 16 NTU compared to the previous week. Increased runoff could carry eroded sediment into the water column of the ponds. Increased wind speeds during precipitation events could resuspend sediment from the pond bottom within the water column (Cech, 2010) but, if occurring, these factors were not enough to significantly impact turbidity in the pond water.

Considerations for Future Research

In future research, pond surface area should be calculated using satellite imaging to obtain accurate measurements of irregularly shaped ponds. More accurate pond measurements will assure the correct mass of straw is added. Straw should be placed in ponds by mid-May to insure decomposition of straw and production of inhibitory chemicals have ample time to commence before algal populations begin to grow. The sampling transect should be established in the windward to leeward direction of each pond to control for movement of algae by wind. Variability among ponds should be controlled by building microcosms within a singular pond according to the method of Baker et al. (2018). The Baker et al. (2018) approach would allow for all three treatments to be compared within one ecosystem under the same abiotic conditions. Microbial community composition analysis would provide insight into food web dynamics and growth inhibition of specific algal species. Using dissolved oxygen and temperature sensors that constantly record data in each pond to determine daily maximums and minimums could account for variability in measurements resulting from the time of day when samples are collected. In-vitro testing in microcosms with sterilized pond water and inoculation with known algal species would allow for investigation of mechanisms responsible for inhibition and abiotic factors influencing outcomes in pond water under controlled conditions, eliminating multiple sources contributing to variability in results.

Conclusion

Due to the lack of significant differences in chlorophyll-a among the treatments and control, neither rice straw nor barley straw were effective at inhibiting algal growth in the farm ponds studied. Since there was no difference between the rice straw and barley straw treatments, it is unclear if rice straw is as effective as barley straw at inhibiting algal growth. Further research is needed to determine the efficacy of cereal grain straw as a reliable method of algal biomass control.

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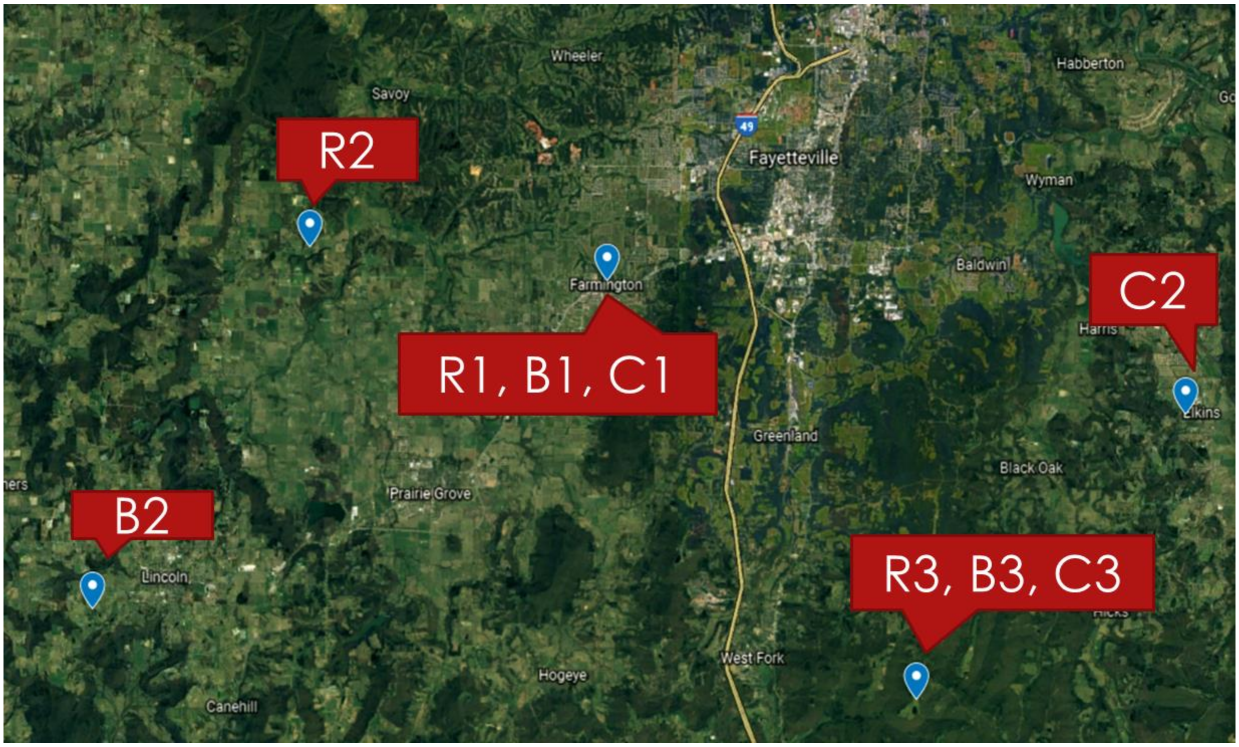

Figure 1. Map from Google Earth (2019) showing the location of each pond studied in Washington County. Ponds R1, R2, and R3 were treated with rice straw. Ponds B1, B2, and B3 were treated with barley straw. Ponds C1, C2, and C3 were left untreated as a control.

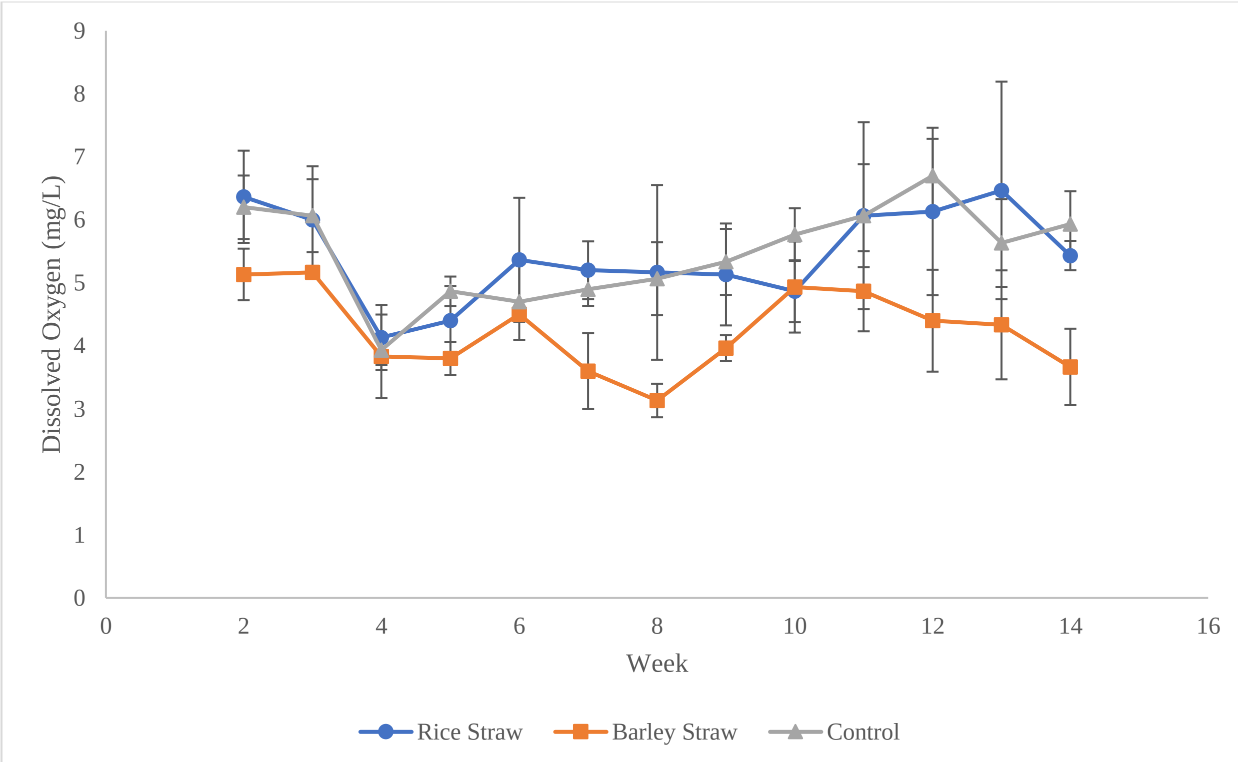

Figure 2. Dissolved oxygen concentrations (mg/L) in ponds treated with rice straw, barley straw, and no treatment (control) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples for each treatment were averaged (n = 3). Error bars depict standard error of the mean.

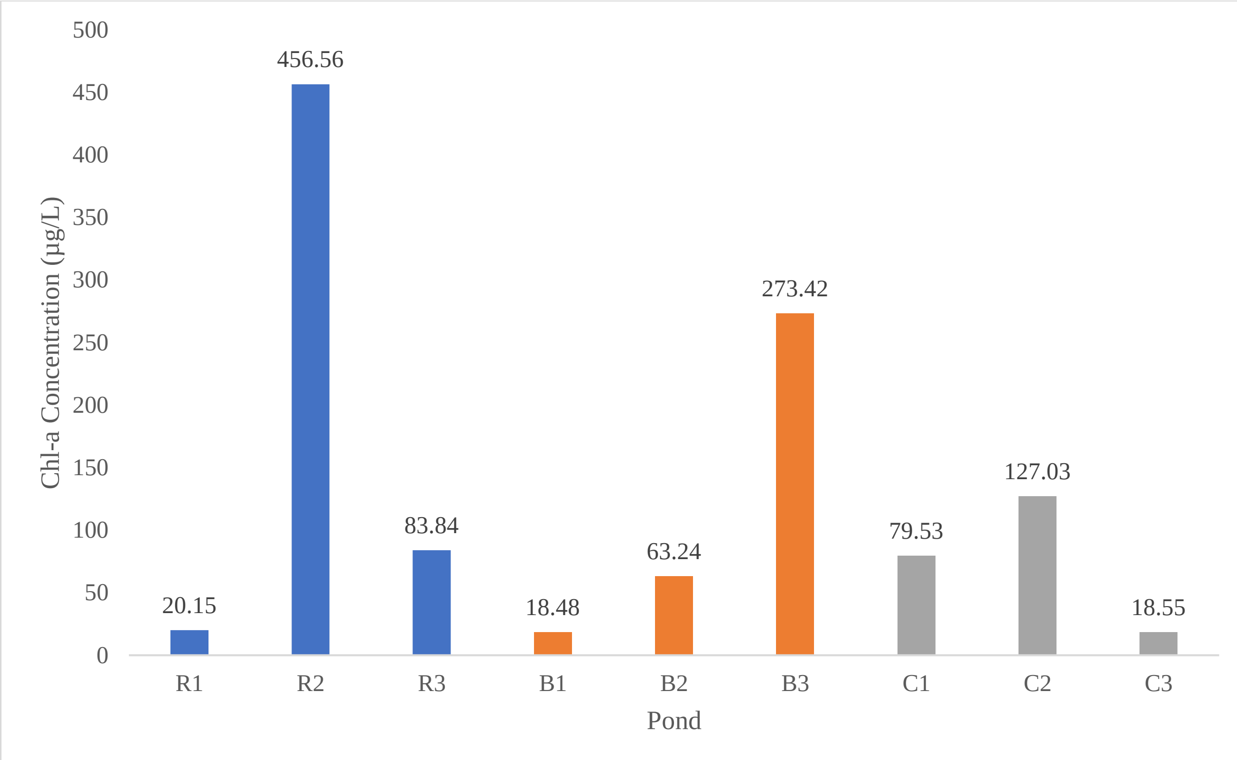

Figure 3. The initial chlorophyll-a (Chl-a) concentration (µg/L) in each pond on June 12, 2018 when straw was placed in the ponds. Ponds R1, R2, and R3 were treated with rice straw. Ponds B1, B2, and B3 were treated with barley straw. Ponds C1, C2, and C3 were left untreated as a control. Numbers above the bars are the initial chlorophyll-a concentration in each pond.

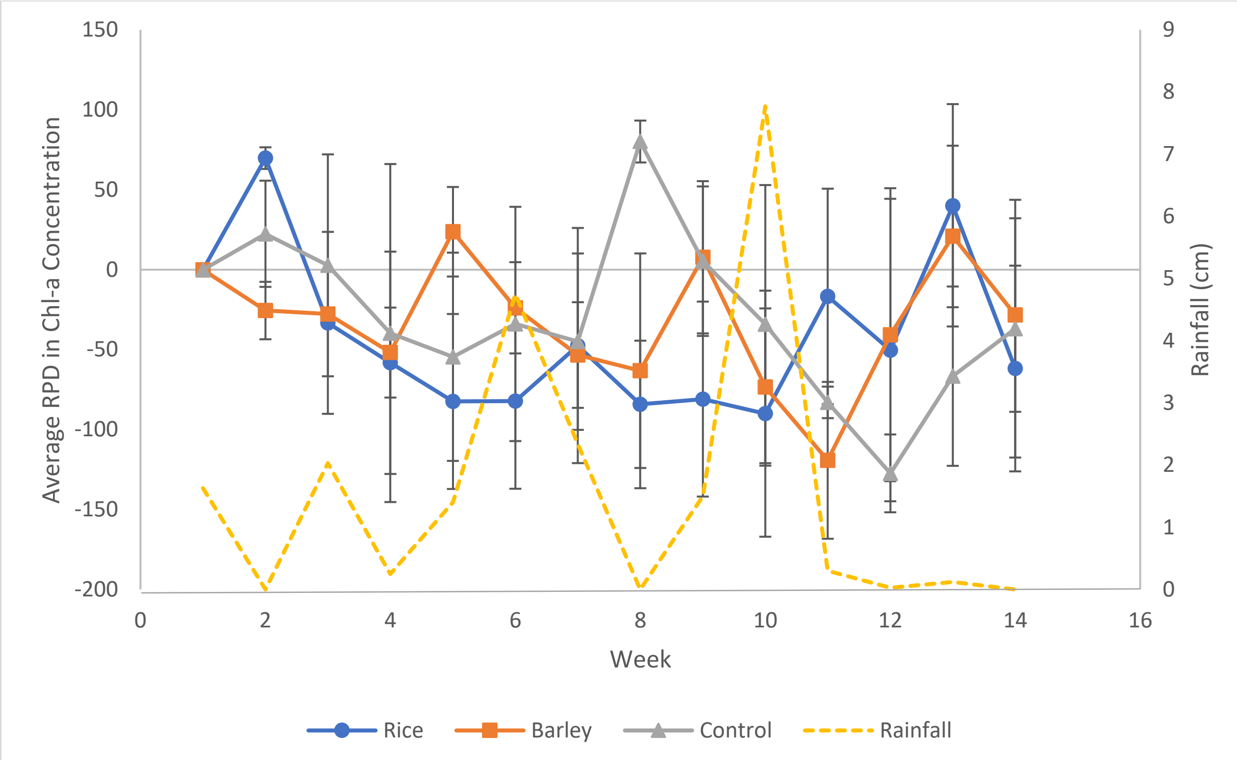

Figure 4. Relative percent difference (RPD) from week 1 of chlorophyll-a concentrations (µg/L) in ponds treated with rice straw, barley straw, and no treatment (control) with weekly rainfall data in cm (United States Geological Survey, 2018) during the 14-week study (June 12, 2018 to September 17, 2018). Samples for each treatment were averaged (n = 3). Error bars depict standard error of the mean.

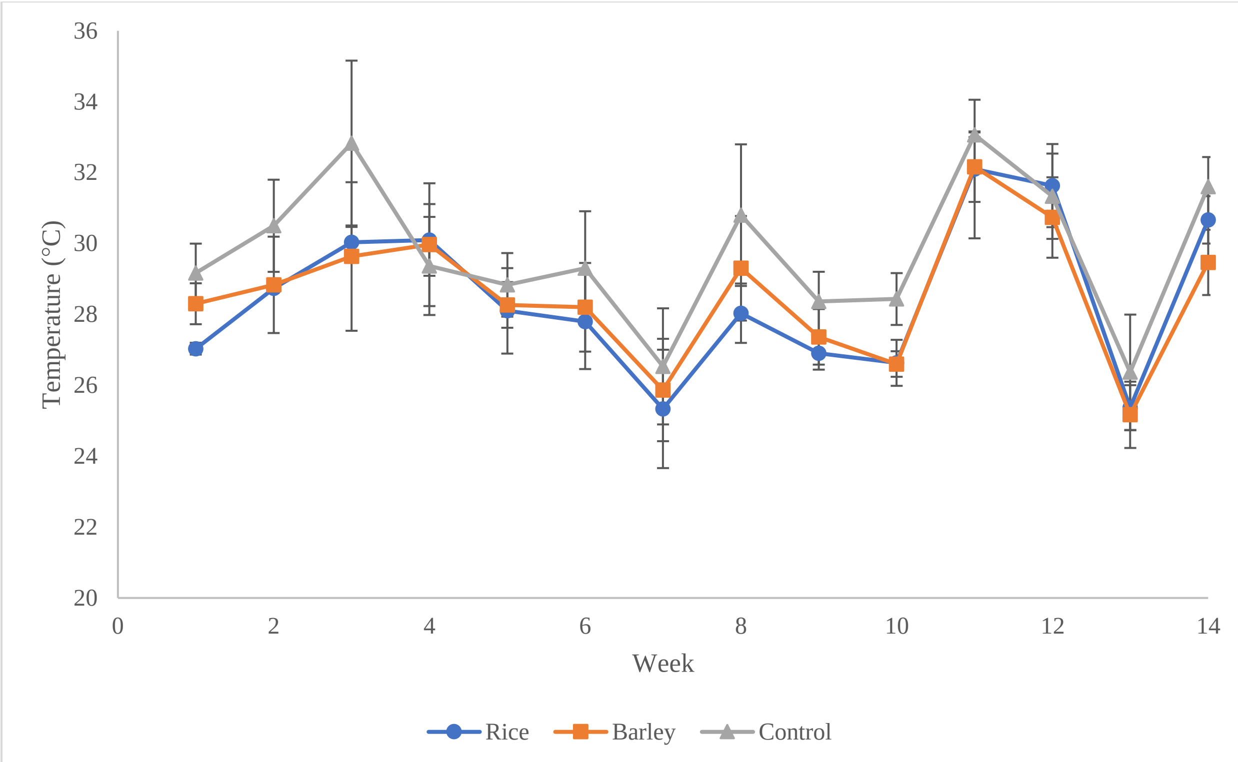

Figure 5. Temperature (°C) in ponds treated with rice straw, barley straw, and no treatment (control) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples for each treatment were averaged (n = 3). Error bars depict standard error of the mean.

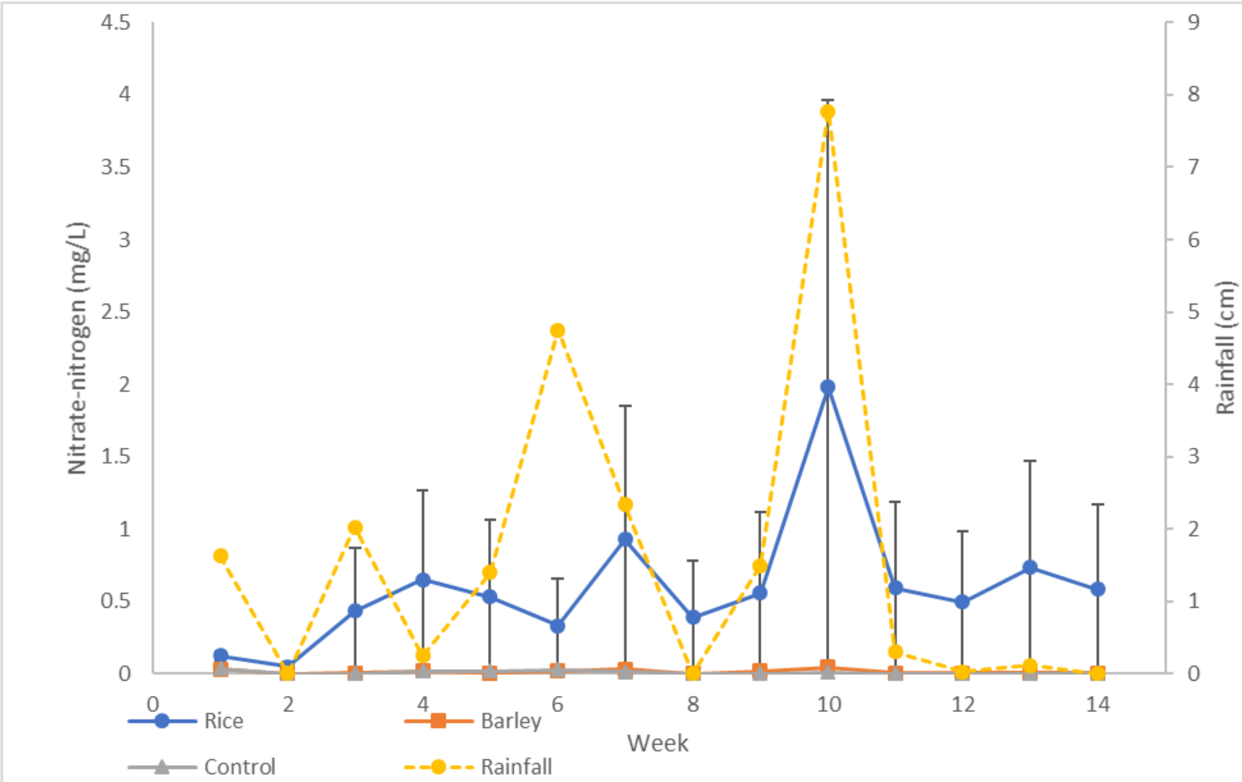

Figure 6. Nitrate-nitrogen concentrations (mg/L) in ponds treated with rice straw, barley straw, and the control (no treatment) with weekly rainfall data in cm (United States Geological Survey, AR, 2018) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples for each treatment were averaged (n = 3). Error bars are standard error of the mean.

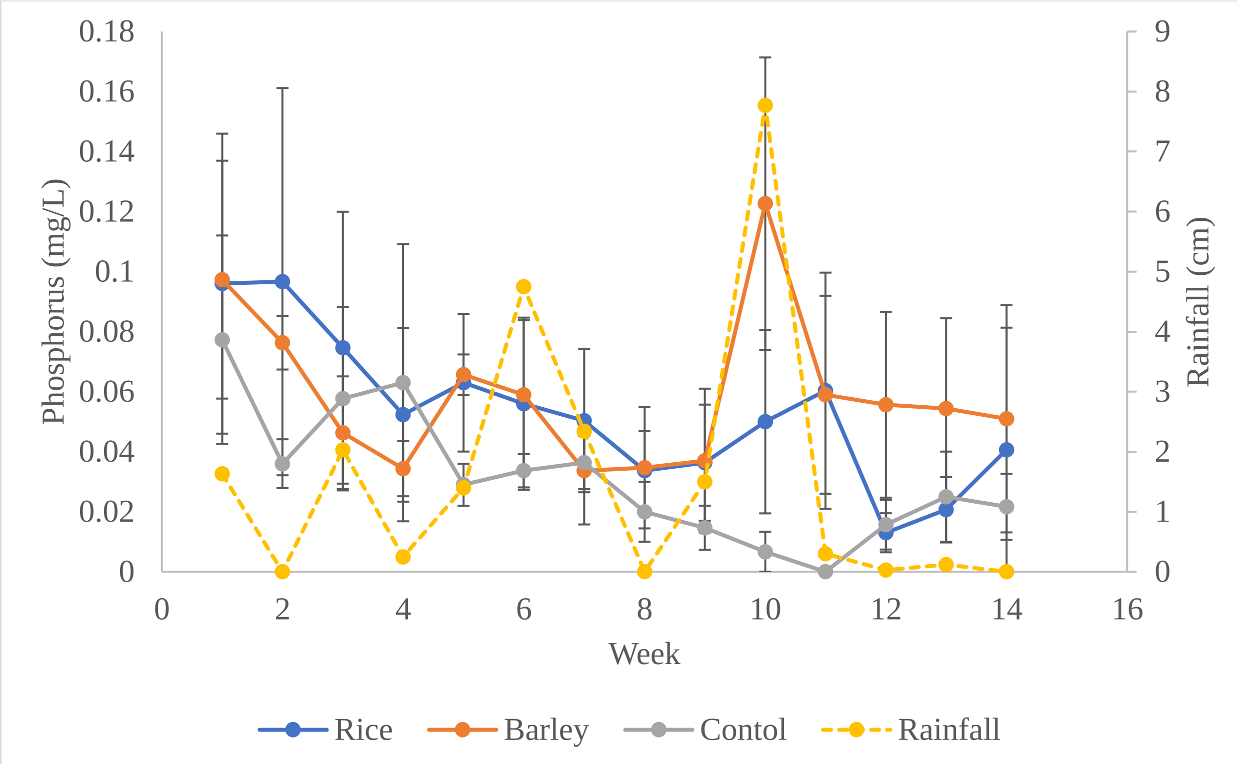

Figure 7. Dissolved phosphorus concentrations (mg/L) in ponds treated with rice straw, barley straw, and the control (no treatment) with weekly rainfall data in cm (United States Geological Survey, AR, 2018) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples for each treatment were averaged (n = 3). Error bars are standard error of the mean.

Table 1. City name, GPS coordinates, surface area, mass of straw added, and the surrounding land use of each pond in the study. Ponds R1, R2, and R3 were treated with rice straw. Ponds B1, B2, and B3 were treated with barley straw. Ponds C1, C2, and C3 were left untreated as a control.

Pond ID	City	Global Positioning System (GPS) Coordinates for each Pond	Surface Area (m^2)	Straw Added (kg)	Surrounding Land Use
R1	Farmington, AR	36°01'49.6"N 94°14'16.9"W	230.8	5.770	Horse and donkey pasture with access to pond Occasional cattle
R2	Farmington, AR	36°03'12.2"N 94°21'45.2"W	670.7	16.768	Cattle pasture with access to pond Domestic ducks and geese nesting on pond bank
R3	West Fork, AR	35°54'42.7"N 94°07'22.5"W	1514.9	37.870	Cattle pasture with access to pond Occasional wild ducks in pond
B1	Farmington, AR	36°01'53.8"N 94°14'17.5"W	414.8	10.369	Horse and donkey pasture with access to pond Occasional cattle
B2	Lincoln, AR	35°56'19.1"N 94°27'08.1"W	286.5	70.911	Cattle pasture with access to pond
B3	West Fork, AR	35°54'45.0"N 94°07'32.2"W	1631.8	40.795	Cattle pasture with access to pond
C1	Farmington, AR	36°01'55.4"N 94°14'11.4"W	1436.6	0	Horse and donkey pasture with access to pond
C2	Elkins, AR	36°00'02.3"N 94°00'53.3"W	2251.4	0	Cattle pasture with access to pond Ranging chickens
C3	West Fork, AR	35°54'22.6"N 94°07'35.8"W	2302.7	0	Cattle pasture with access to pond

Table 2. Analysis of variance summary of the effects of straw treatment and time on properties measured in pond water.

Dependent variable	*P*-value		
	Straw treatment	Time	Straw treatment by time
Chlorophyll-a	0.845	0.694	0.909
Dissolved oxygen	$2.9x10^{-5}$**	0.019*	0.997
Temperature	0.009*	$2.85x10^{-10}$**	0.998
NO_3^--N	0.0003**	0.976	0.998
Phosphorus	0.024*	0.274	0.971
pH	0.090	0.202	0.890
Turbidity	0.081	0.466	0.660

*P < 0.05; **P < 0.001

Appendix 1

Introduction

A preliminary trial of this study was conducted in the fall of 2017 at the Botanical Garden of the Ozarks in Fayetteville, AR as a part of the Plants and Environmental Restoration course at the University of Arkansas. Data were collected by a group of five students and presented at the end of the semester to the Botanical Garden of the Ozarks staff.

Research Question

1. Is barley straw effective at inhibiting algal growth in small ponds at the Botanical Garden of the Ozarks?
2. Is rice straw an adequate substitute for barley straw in algal bloom inhibition?

Objective

The objective of this small-scale, preliminary study was to determine procedures for an experimental approach to test the effectiveness of rice straw compared to barley straw at preventing the growth of algae when allowed to decompose aerobically in ponds.

Prediction

Algal growth, measured as chlorophyll-a, was expected to be reduced in ponds with rice and barley straw when compared to an untreated control. Rice straw was expected to be as effective as barley straw at preventing the growth of algal populations, measured as chlorophyll-a.

Materials and Methods

Experimental Setup

There are three ponds located at the Botanical Garden of the Ozarks (the Front Pond, the Japanese Garden Pond, and the Children's Garden Pond) in Fayetteville, AR. Each pond was randomly assigned to one of three treatments. The Japanese Garden Pond was treated with rice straw, the Front Pond was treated with barley straw, and the Children's Garden Pond was left untreated as a control. The surface area of the three ponds was calculated by measuring the length and width of each pond with a tape measure (Table A1). Barley straw was purchased as pre-portioned bags marketed as pond amendments (Summit Chemical Inc, Baltimore, MD) and rice straw was obtained from the University of Arkansas Research Station in Stuttgart, Arkansas. Ponds were treated at a rate of 22.45 g/m^3. The appropriate mass of rice straw was portioned into a bag with 0.5-cm mesh opening of similar dimensions to the pre-bagged barley straw (approximately 20 cm by 20 cm by 40 cm). Straw bags were placed in the filter of the Front Pond and Japanese Garden Pond, so the bags would be well aerated, and all water would contact the decomposing straw. Straw bags were placed in ponds on October 5, 2017.

Sampling

Beginning on the date of straw placement (October 5, 2017), 1-L water samples were collected weekly from each pond for seven weeks. Samples were taken at a depth of 15 cm by reaching to the center of each pond while standing on the edge of the pond. Samples were immediately covered in aluminum foil to prevent further photosynthesis and photo degradation. The final samples was were collected on November 16, 2017.

Chlorophyll-a

From the 1 L sample, 50 mL was filtered in the field using a hand pump and GF/F filter (Whatman, 0.7-µm pore size). Filtrate was discarded. This was done three times for each pond sample for a total of nine filters. After returning to the laboratory, each filter was soaked in 7 mL of 90% acetone for 24 hours and stored in a freezer. The extract was analyzed using a Trilogy Laboratory Fluorometer (Turner Designs, San Jose, CA). The "Chl-a" module of the fluorometer was calibrated using a stored calibration curve. After samples had been equilibrated to room temperature, extract from each sample (3 mL) was pipetted into a culture tube. Each tube was placed into the fluorometer one at a time. The sample was measured before acidification. After the measurement was complete, 0.1 mL of 0.1 N hydrochloric acid was pipetted into the tube. Following a 90-second reaction period, the sample was measured after acidification. The acidification step converted all chlorophyll-a to pheophytin, a degradation product of chlorophyll-a, and a pheophytin-corrected chlorophyll-a concentration (µg/L) was displayed and concentrations were recorded.

Water Quality Parameters

Using the remaining 850 mL of the water sample, turbidity, pH, dissolved oxygen, nitrate-nitrogen (NO_3^--N), and phosphate (PO_4^{3-}) were measured using a LaMotte Freshwater Aquaculture Test Kit (Carolina Biological Supply Company, Burlington, NC). Three measurements were taken weekly from each water samples.

Data Analysis

Averages, standard deviations (α), and standard error of the means (equation shown below) were calculated each week for chlorophyll-a, NO_3^--N, phosphorus, dissolved oxygen, pH, temperature, and turbidity.

- $SE = \frac{\alpha}{\sqrt{n}}$

Data organization, graph creation, and data analysis were conducted in Excel 2016 (Microsoft Corp., Redmond, WA). Repeated measures analysis of variance (ANOVA) tests were performed on each dependent variable to determine statistical significance at $\alpha = 0.05$. Statistical analyses were used to determine if dependent variables differed across treatments over time.

Results and Discussion

Averaged across time, chlorophyll-a concentrations did not differ statistically among treatments and the control ($P = 0.492$). Chlorophyll-a concentrations were low in all three ponds, ranging from 1.35 to 3.72 μg/L in the Japanese Garden Pond treated with rice, ranging from 1.62 to 3.85 μg/L in the Front Pond treated with barley, and ranging from 1.41 to 3.48 μg/L in the Children's Garden Pond without treatment. Low chlorophyll-a concentrations and absence of statistical treatment significance may have been a result of the time of year the study was conducted. The study began in October when seasonal air temperatures and daylight hours decline which is less ideal for algal growth.

The PO_4^{3-} and NO_3^--N La Motte tests failed to produce a value higher than 0 mg/L in any treatment during the study. The LaMotte test kit measured PO_4^{3-} and NO_3^--N to 0.1 mg/L; thus, varying nutrient concentrations below 0.1 mg/L could not be detected. Since the study was conducted during autumn and after the growing season of most plants, the Botanical Garden of the Ozarks may not have been applying fertilizer at the time of the study which could result in less nutrient input into the ponds than might occur during the spring and summer.

Averaged across time, pH did not differ statistically among treatments and the control ($P = 0.262$). The pH ranged from 7.5 to 8.5 in the Japanese Garden Pond (treated with rice straw) and Children's Garden Pond (control) and from 8 to 8.5 in the Front Pond (treated with barley straw). The slightly basic pH of the ponds might coincide with high algal biomass concentration since large algal populations raise pH (National Oceanic and Atmospheric Administration, 2017); however, this relationship was not supported by the chlorophyll-a data. Instead, the basic pH may have resulted from water hardness from the well water containing calcium carbonate, but neither the water hardness nor the well water specifically was measured (Cech, 2010). The LaMotte test kit measured pH to a precision of 0.5 pH unit. There may have been greater variability among measurements that could not be discerned by the precision of the kit.

Averaged across time, dissolved oxygen did not differ statistically among treatments and the control ($P = 0.739$). Dissolved oxygen ranged in the Japanese Garden Pond (treated with rice straw) from 6.8 to 9.5 mg/L, in the Front Pond (treated with barley straw) from 6.8 to 10 mg/L, and in the Children's Garden Pond (no treatment) from 7.2 to 10 mg/L. The lack of a difference in dissolved oxygen between treatments suggests that the oxygen utilized by microbes to decompose the rice and barley straw was not enough to negatively affect the dissolved oxygen concentration of the pond system. All three ponds were sampled at the same time of day, so variability from photosynthesis and respiration during the day should not have affected the results. The average dissolved oxygen concentrations remained at or above 6.8 mg/L, which is sufficient for aerobic decomposition to occur (Cech, 2010).

The LaMotte turbidity test never produced a value greater than 0 Nephelometric Turbidity Units (NTU). Well managed, thickly vegetated land around ponds could have prevented soil erosion and deposition in the ponds. The ponds also had rubber liners with little substrate, so there was little sediment present in the pond systems. A different method of measuring turbidity with greater sensitivity may have produced different, more accurate results.

In a larger-scale version of this study, a different source of barley straw should be used. The pre-packaged bags of barley straw are produced for small ponds. The pre-packaged straw is also sold in only a few masses, so purchasing the exact mass of straw needed for the surface area of ponds is not always possible. Purchasing barley straw in bulk and bagging it before placement would be less expensive and allow for any specific mass of straw to be added to ponds. More sensitive and accurate methods for analyzing nutrients, pH, turbidity, and dissolved oxygen should be used to obtain reliable results for small concentrations. Water temperature should be recorded weekly as well. The time period of the study should incorporate the months of the algal growing season.

Conclusions

Due to the lack of significant differences in chlorophyll-a among the treatments and control, neither rice straw nor barley straw were effective at inhibiting algal growth in the ponds studied. However, this result may be attributed to the time of year that the study occurred (autumn) rather than as a result of the treatments.

Table A1. Surface area, weight of straw added, and the surrounding land use of each pond at the Botanical Garden of the Ozarks. The Japanese Garden Pond was treated with rice straw. The Front Pond was treated with barley straw. The Children's Garden Pond was left untreated as a control.

Pond Name	Surface Area (m^3)	Straw Added (kg)	Surrounding land use
Japanese Garden Pond	16.5	0.365	Heavily managed garden
Front Pond	59.6	1.405	Heavily managed garden
Children's Garden Pond	32.8	0	Heavily managed garden

Appendix 2

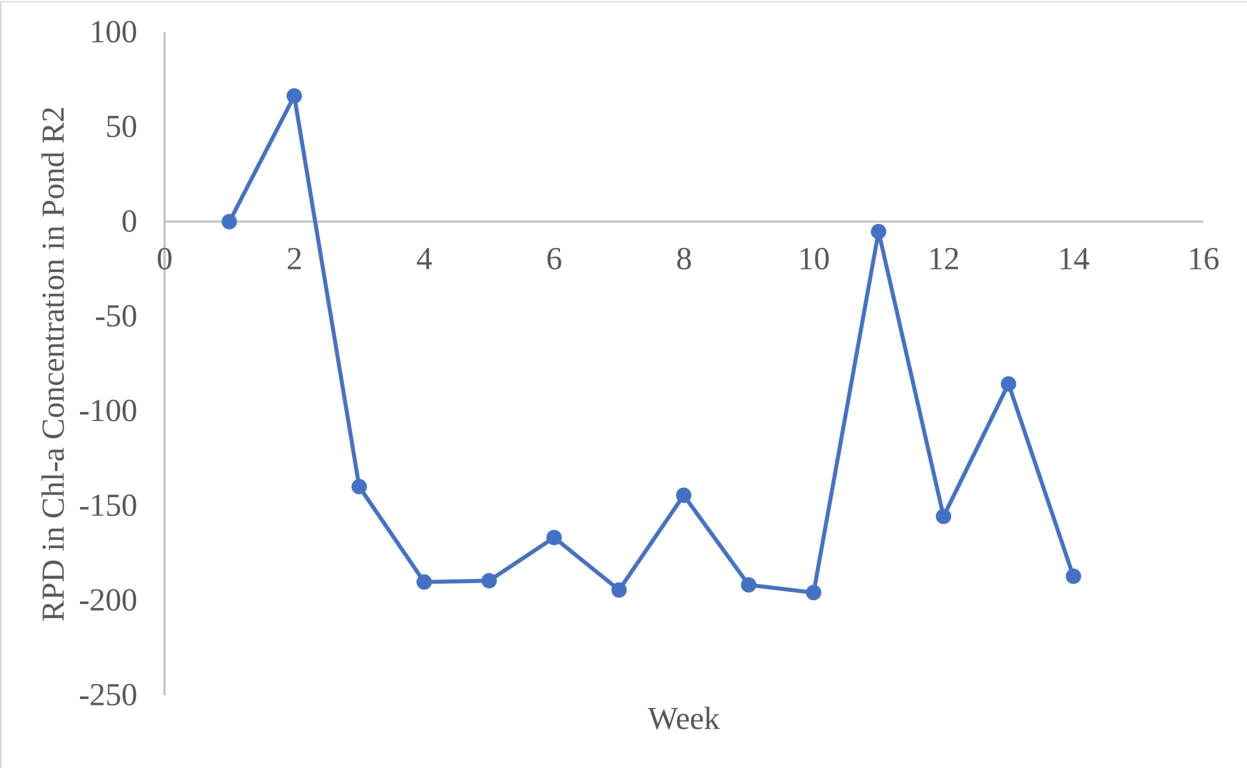

Figure B1. Relative percent difference (RPD) from week 1 of chlorophyll-a concentrations (μg/L) in pond R2 (treated with rice) during the 14-week study (June 12, 2018 to September 17, 2018).

Appendix 3

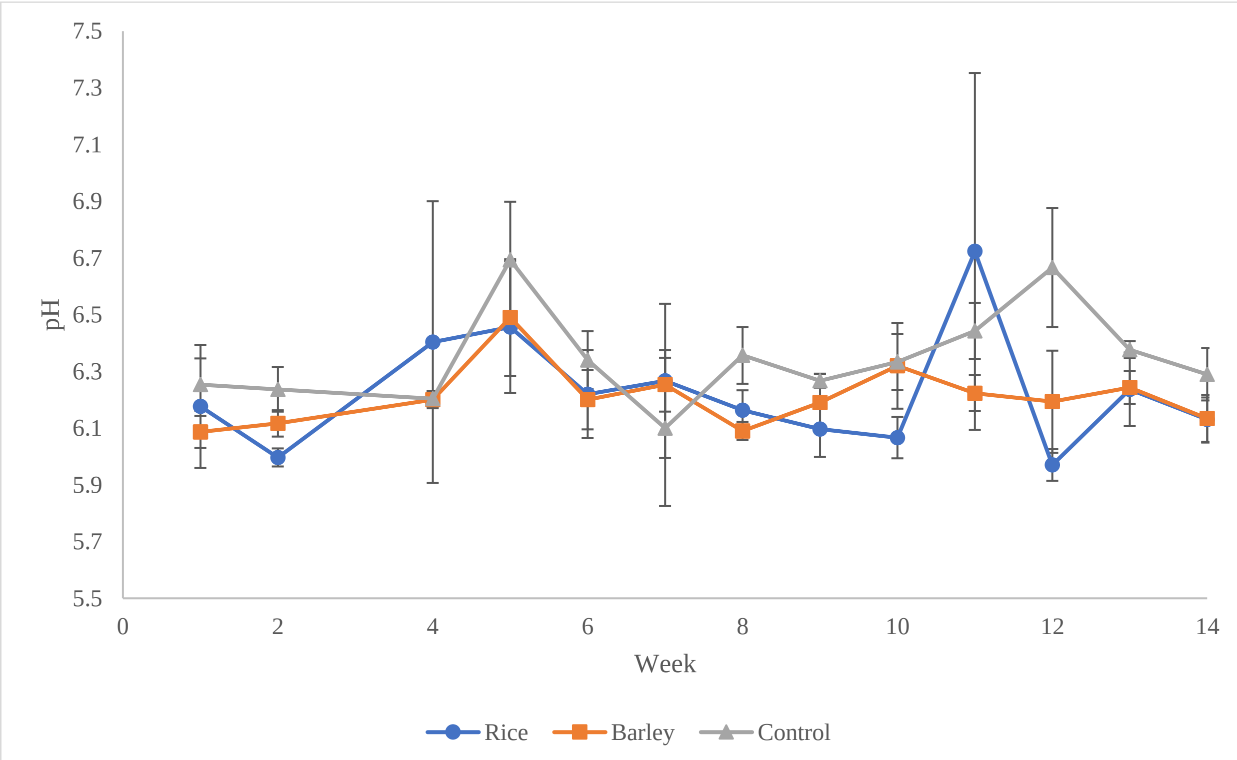

Figure B2. The pH in ponds treated with rice straw, barley straw, and no treatment (control) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples in each treatment were averaged (n = 3). Error bars are standard error of the mean.

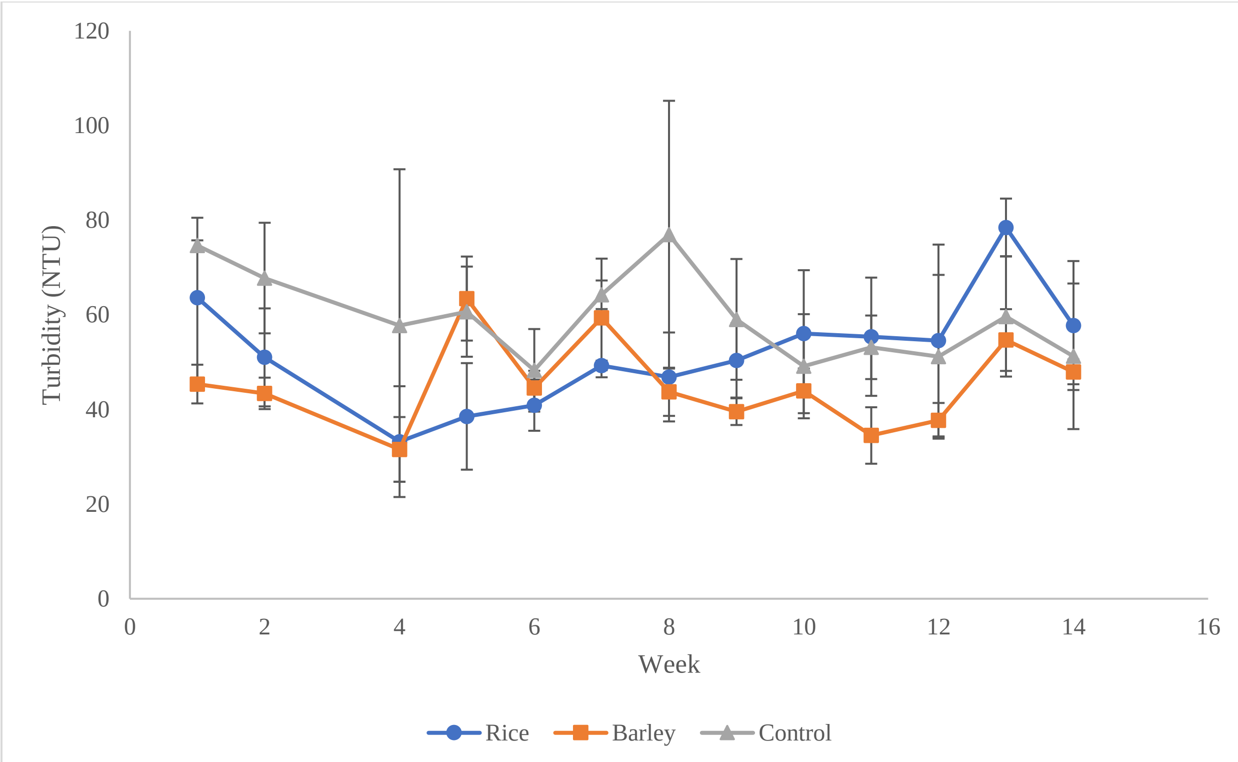

Figure B3. Turbidity in ponds treated with rice straw and barley straw and with no treatment (control) during the fourteen-week study from June 12, 2018 to September 17, 2018. Samples in each treatment were averaged (n = 3). Error bars depict standard error of the mean.